FINAL REPORT

GERM CELL MUTAGENESIS in MEDAKA FISH FOLLOWING EXPOSURE to HEAVY, HIGH ENERY COSMIC RAY NUCLEI

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NASA Grant Number: T-5270W

July 26, 2003

Introduction:

A significant hazard to astronauts going beyond low Earth orbit results from the presence of ionizing radiations, in particular **High Energy, High Atomic Number Nuclei (HZE)** Particles, such as 56 GeV ^{56}Fe nuclei. These particles produce dense tracks of ionizations in passing through tissue and, as a result, will damage the genetic material of cells, DNA. The result may be the killing of cells or the induction of mutations that could lead to the initiation of cancers or to deleterious mutations in germ cells (sperm or oocytes) that could be transmitted to progeny. Germ cell mutations in astronauts could result in mutations in their offspring that could render them less fit to cope with the normal hazards in the world.

The radiobiological effects of Low Energy Transfer (LET) radiations such as X rays and γ rays are well known. The carcinogenic effects of LET radiations are well estimated from the observed effects of the nuclear bombs on the Japanese survivors. However, although no significant increases above background mutations among the progeny of exposed individuals have been observed, their magnitudes may be estimated by extrapolation from so called mouse specific locus tests (SLT). Such tests observe mutations, usually coat color mutants, in the progeny of exposed males. The tests on mice have not been carried out using HZE nuclei because the large numbers of offspring needed to detect the low frequencies of SL mutations makes these tests impossibly laborious. A relatively new, quantitative SLT has been developed by the Japanese Co-PIs. It uses Medaka fish (*Oryzias latipes)*, ~2-3 cm long.

Materials and Methods:

The general design of the experiments is to expose wild type males to radiations of known LET and mate them individually with a female that is homozygous recessive for 5 color markers. The virtue of this system is that the fish are oviparous and the transparent eggs (embryos) may be observed during their development over the 6-9 day period before hatching. As a result, embryos destined to die before hatching may be scored for dominant lethal (DL) and specific color (SL) mutations, increasing by orders of magnitude the number of observable mutations compared to mutations in viable progeny. Extensive quantitative data exist relating mutation frequencies to γ-ray doses (low LET) to sperm [embryos collected 1-3 days following exposure], to spermatids (tids) [embryos collected 4-9 days following exposures] or to spermatogonia (gonia) [embryos collected 32 or 46-117 days after exposure]. The dose response relations are linear for both DL and SL mutations following exposures at all stages of spermatogenesis [Shimada and Shima, Mutation Res. **399** (1998) 149-165].

On two separate occasions (Exp. 1 in Feb. 2001 and Exp. 2 in Apr. 2002) ~80 male Medaka were sent, via air express, from Japan to the Brookhaven National Laboratory (BNL). After a few days of acclimatization, they were exposed, 10 at a time in T25 flasks, to 1 Gy/min of a 1 GeV/nucleon beam of ^{56}Fe nuclei at the Alternating Gradient Synchrotron at BNL. After exposures of 0.3 and 1.0 Gy in 2001 and 1.0 and 2.0 Gy in 2002, the fish were returned to Japan within a day. There, the embryos (10-15/day) from individual mating pairs were collected individually into the wells of 96-well microtiter plates and scored daily for DL and SL mutations, both total (TM) and viable (VM).

Results:

Table 1 shows the numbers of embryos collected and scored from historical

Table 1: Numbers of Embryos Scored[1]

Exp. #	Dose (Number)	Stage[2] Exposed	Embryos Collected	Fraction Fertilized	Embryos Developed	Effective Loci (10^4)
Historical Controls					27870	12.2
1	0.3 Gy	sperm	2131	0.84	1698	0.84
	(48)	tids	4770	0.87	4024	2.00
		gonia	13,757	0.86	11288	5.89
1	1.0 Gy	sperm	1636	0.86	1353	0.67
	(36)	tids	3660	0.82	2925	1.45
		gonia	10459	0.83	8411	4.32
2	1.0 Gy	sperm	1740	0.69	1200	0.60
	(38)	tids	4793	0.73	3436	1.71
		gonia	17869	0.47	8359	4.16
2	2.0 Gy	sperm	2314	0.67	1466	0.72
	(40)	tids	4517	0.69	3122	1.54
		gonia	22300	0.33	7368	3.64

[1] Matings of exposed (56 GeV ^{56}Fe nuclei) wild type males with unexposed females with recessive color mutations at 5 loci.
[2] Matings of exposed sperm, days 1-3; spermatids (tids), days 4-9; spermatogonia (gonia), days 32-149 for Exp.#1 and days 46-117 for Exp.#2.

controls and that the numbers from the individual doses in the two experiments ranged from 1640 - 2300 for exposed sperm, 3660 – 4790 for exposed tids and 10500 – 22300 for exposed gonia. The fertilization rates were ~0.85 in 2001 and, in 2002, ~0.70 for sperm and tids and ~0.4 for gonia. The numbers of early deaths within 24 hr were between 1-5% and were independent of dose, presumably a maternal effect, and were not included in the calculations of DL. Table 1 also shows the numbers of embryos that developed and the numbers of effective mutagenic loci for the various exposures.

Table 2 gives the numbers of dead embryos (dominant lethal mutants, DL), total

Table 2: Numbers of Mutations Observed

Exp.#	Dose	Stage	Dead	TM[1]	VM[2]
Controls	0.00		700	4	1
1	0.3 Gy	sperm	110	7	1
		tids	191	12	0
		gonia	520	11	1
1	1.0 Gy	sperm	143	19	1
		tids	276	43	0
		gonia	382	13	1
2	1.0 Gy	sperm	132	16	0
		tids	291	45	2
		gonia	318	2	1
2	2.0 Gy	sperm	284	43	0
		tids	548	78	5
		gonia	287	2	1

[1] TM: Total Mutations
[2] VM: Viable Mutations

mutants (TM) and viable mutants (VM) observed in the individual experiments described in Table 1. There is good agreement in the values of DL for all exposed stages for the two exposures of 1 Gy. However, the values for TM from gonia exposed to 1 and 2 Gy are not consistent. The reason(s) for the inconsistency is not known, but could be related to the low fertilization efficiency for these gonia exposures in Exp.2 (see Table 1). The numbers of VM, for all stages exposed and all doses, is ~1, a number with an uncertainty of 1. Although doses = or >0.3 Gy from 56 GeV Fe nuclei result in observable color mutations in developing embryos, the embryos do not survive. Hence, the frequencies of VM mutations will not be tabulated.

Table 3 gives the mutation frequencies calculated from the data in Tables 1 and 2.

Table 3: Mutation Frequencies Observed

Exp.#	Dose	Stage	DL Frequency[1]	TM Frequency[2] (10^{-4})
Controls	0.00		0.025	0.33
1	0.3 Gy	sperm	0.065	8.3
		tids	0.048	6.0
		gonia	0.046	1.9
1	1.0 Gy	sperm	0.106	28.
		tids	0.094	30
		gonia	0.045	3.0
2	1.0 Gy	sperm	0.110	27
		tids	0.085	26
		gonia	0.038	0.48
2	2.0 Gy	sperm	0.194	59
		tids	0.176	51
		gonia	0.039	0.55

[1] Dominant Mutation Frequency per Embryo.
[2] Total Mutation Frequency per Locus.

The inconsistency in the TM frequencies for exposed gonia is apparent. For exposed sperm and tids, the frequencies increase with dose in a linear fashion as indicted in Fig. 1 (DL) and Fig. 2 (TM).

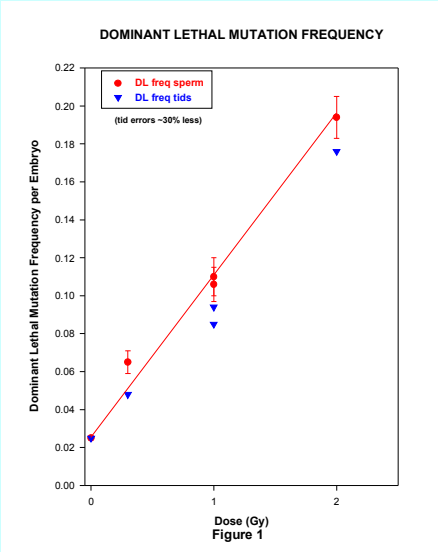

Figure 1

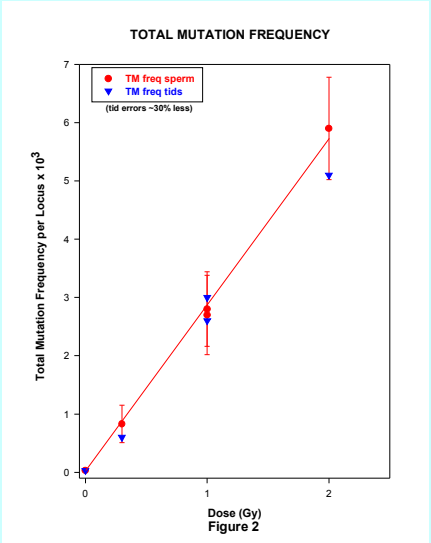

Figure 2

Discussion:

There are extensive dose-response data for DL and TM for exposures to γ rays. As in Figs.1 and 2, the mutation frequencies increase linearly with dose. The ratio of the slope of the dose response curve (mutation frequency per unit dose) for exposures to 56 GeV Fe nuclei to the slope for γ rays give the Relative Biological Effectiveness (RBE) of the Fe nuclei. The results of these calculations are given in Table 4. The precision in the

Table 4: RELATIVE BIOLOGICAL EFFECTIVENESS* OF 56 GeV Fe NUCLEI, by Stage Exposed

	Sperm	Spermatids	Spermatogonia**
Dominant Lethals	**1.3**	**1.2**	**~4**
Total Mutations	**1.9**	**4.0**	**~4**

*Relative to historical γ-ray data, (Shimada & Shima, 1998) assuming linear dose-response relations passing through the 0.00 Gy data point.

**Only from Exp.1 (Exp. 2 inconsistent, Table 2)

values of RBE is ~10% for the values of DL and TM for exposed sperm and spermatids, but only ~50% for exposed spermatogonia. The noteworthy feature of these RBE values at an LET of 147 keV/μm, is that they are ~10 fold, or more, smaller than those obtained for the few studies on cell transformation and cancer induction in rodents [See Fig. 7 in F. A Cucinotta, et al. "Space Radiation Cancer Risk Projections for Exploratory Missions: Uncertainty Reduction and Mitigation", NASA JSC Document (JSC-29295) 2001.]

Conclusions:

1. The RBE values for germ-cell mutations are ~10-fold less than those for cancer induction or the killing/mutation of somatic cells.
2. The numbers of viable mutations from all exposed germ-cell stages are very low and would not yield significant numbers of mutations above background levels.
3. Total mutations from exposed spermatogonia show barely significant increases above control levels.
4. Stem cells damaged by exposures to High LET cosmic rays probably undergo apoptosis or result in abnormal sperm that would yield large fractions of dominant lethal mutations.
5. The hazard to male astronauts from exposures to high LET cosmic rays is probably temporary sterility, but not significant mutagenic effects observable in progeny.

Other Information:

Abstracts-Proceedings

1. Setlow, R. B., Shima, A. and Shimada, A. "Germ-Cell Mutagnesis by HZE Nuclei: Preliminary Results". Bioastronautics Investigators Workshop, January 17-19, 2001.
2. Setlow, R. B., Shimada, A. and Shima, A. "Germ-Cell Mutagenesis by HZE Nuclei: RBE Values. Bioastronautics Investigators Workshop, January 13-15, 2003.